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# Effect of simulated long transport on behavioural characteristics in two strains of laying hen chicks

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## Abstract

Newly hatched chicks are routinely subjected to varying durations of transport shortly after hatching. Because little is known about the effects of this putative stressor on behavioural development, the present experiment tested for the effects of a simulated long transport-like treatment during the first day of life in two strains of laying hen chicks on growth, the development of perching behaviour, fear of humans (measured as the duration of tonic immobility induced by manual restraint) and the willingness to compete for access to feed. Fourty newly hatched Hy-Line W-36 (W-36) and 40 Hy-Line Brown (HB) chicks distributed over two blocks were subjected either to a short duration of handling and transport prior to delivery at the laboratory (SHORT treatment; duration of 4 h) or to the same treatment plus an additional period of intermittent movement used to simulate longer transport (LONG treatment; duration of 14 h).

LONG birds started perching at a significantly earlier age than SHORT birds ($P < 0.01$), but strain effects on the duration or frequency of perching were non-existent or sporadic. W-36 birds had a longer duration of tonic immobility than HB birds ($P < 0.001$) but there was no effect of the treatment ($P > 0.10$). The treatment reduced the birds' willingness to compete for feed ($P < 0.02$), but this was mainly due to an effect on W-36 birds, as indicated by an interaction between treatment and bird strain ($P < 0.02$). The results thus indicate that the LONG treatment reduced the age at which birds first used perches and reduced the willingness to compete for feed. However, as these results were rather sporadic we could not show that the long transport-like treatment of day old chicks has wide-reaching effects on

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behavioural development. Further studies are needed to assess the long-term effect of transport duration on chicks and laying hens.


*Keywords:* Transport; Stress; Chickens; Perches; Competition; Fear

## 1. Introduction

A large body of research in chickens indicates that the early environment is crucial for the development of later behaviour. Different studies document effects of exposure to novelty (Jones, 1997), human contact or handling (Grigor et al., 1995; Fluck et al., 1997), environmental enrichment (Jones, 1982), cold stress (Davis and Fischer, 1978), mechanical restraint (Marin et al., 2001) and access to perches (Gunnarsson et al., 2000). However, there is no information about how exposure to long durations of transport in day old chicks might affect their subsequent behaviour. This is important because conventional layer and broiler chicks are handled in connection with sorting, sexing, immunization and packaging, and then transported over rather long distances for delivery to the farm. According to current EU legislation pullets can be transported for a maximum of 24 h and chicks may be held without access to feed or water for up to 72 h post-hatch. Although this legislation is based on the fact that chicks can survive on metabolic reserves stored in their yolk sack for up to 3 days, it is possible that exposure to long durations of transport without access to feed or water may cause some stress in chicks and adversely affect their development.

Different laying hen strains may differ in their behaviour and the ability to cope with stressors (Klein et al., 2000; Hocking et al., 2004). For example, Webster (1995) found that Hy-Line W77 hens were more adversely affected by a short period of fasting than Hy-Line brown hens, suggesting that these white birds may have a higher sensitivity to metabolic perturbations than the brown birds. Anecdotally, producers often report differences between different lines. They suggest that Hy-Line white hens are more flighty than Hy-Line brown hens and that ISA brown hens have a higher tendency to feather peck than ISA white hens. Brown Hy-Line hens have also been said to be less prone to use perches that white Hy-Line birds. The effects of stress may therefore be expected to depend on the strain's genetic constitution.

The aim of this experiment was therefore to study the effect of the stress caused by a prolonged transport-like treatment on the behaviour of two strains of commercial laying hen chicks. The treatment was meant to provide a standardized application of some of the conditions causing stress during transportation, including prolonged feed and water restriction, novelty, confinement and intermittent movement. The strains were selected from the same breeding company but represent slightly differing breeding goals. The Hy-Line W-36 (W-36) is mainly used for production using cage housing, whereas the Hy-Line Brown (HB) is used for production using both cage and floor systems (http://www.hyline.com/). The W-36 also has a slightly higher feed conversion ratio than the HB (*ibid*), a factor that has previously been shown to influence the ability to cope with stressors (Braastad and Katle, 1989; Beilharz et al., 1993; Beilharz and Mitpaiboon, 1994).

Our arguments for choosing to test for effects of a long duration of simulated transport on growth, fear of humans induced by manual restraint (tonic immobility), the ability to compete for access to feed in hungry chicks and the development of perching behaviour are given in the following. In general we predicted that if the treatment was detrimental to chick development it

should decrease growth rates, increase fearfulness, reduce the ability of chicks to compete and reduce the development of perching behaviour. Growth was chosen as a response variable because stress may inhibit insulin-like growth factor-I secretion and negatively affect growth (Vance et al., 1992; Kakizawa et al., 1995; Bruggeman et al., 1997). We measured the duration of tonic immobility in response to manual restraint because of evidence that the duration of tonic immobility increases in chickens exposed to stress (Jones, 1986), possibly because of exposure to elevated levels of endogenous corticotrophin releasing factor (Adamec et al., 1991). Competitive responses were measured because stress may inhibit the production of gonadal steroids such as testosterone (Rivier and Rivest, 1991) and because low levels of testosterone may attenuate aggressive and competitive responses. Perching is important for the welfare of birds (Gunnarsson et al., 1999, 2000) and depends on coordinated cognitive and motor development (Gunnarsson et al., 2000). Furthermore, previous experiments show that stress may impair cognitive processes (De Kloet et al., 2005; Hage et al., 2006) and could therefore impair the development of normal perching behaviour in chicks.

## 2. Material and methods

### 2.1. Chicks

Forty Hy-Line W-36 (W-36) and 40 Hy-Line Brown chicks were studied in two batches (December 2004 and February 2005). Both batches included 20 chicks of each strain. Due to logistic reasons the batches were bought from two different commercial hatcheries. The chicks were brought from the hatchery on the day they were hatched (day 0), after they were sorted and vaccinated for Marek's disease. The Hy-Line W-36 strain has a higher conversion rate than the Hy-Line Brown (113 g/bird/day versus 92 g/bird/day) (Hy-Line variety W-36 Commercial Management Guide 2003–2005 and Hy-Line Brown Commercial Management Guide 2002–2004).

### 2.2. Housing and care

After transportation the chicks in each batch were randomly divided into eight groups of five chicks, with one treatment × strain combination per pen. The chicks were housed in eight identical pens (75 cm × 75 cm), arranged wall-to-wall in two lines of four pens each. The pens had solid 90 cm high walls, a rounded wooden perch (15 cm high) along the back wall, a feeder and a bell drinker. The perch was long enough to allow all birds to perch simultaneously. Pen order was balanced for treatment and strain. Wood chips were used as litter and were added when necessary to keep a good coverage of the floor. The litter was changed twice during the experimental period, on day 20 and day 36. The chicks were fed with a commercial rearing feed designed for chicks up to 6 weeks of age.

The room temperature was 36 °C when the chicks arrived and was then decreased by 3 °C per week until a temperature of 21 °C was reached. The lighting was fully artificial and lights were on from 09:00 to 19:00 h. In addition, a dimmer light was on from 8:30 to 19:30 h in order to provide an artificial dusk and dawn during which birds could move between perches and the floor. The lights were on continually from the time that the first chicks were put into their pens until 10 h after the last chicks were put into their pens in order to facilitate initial feed intake.

### 2.3. Treatments

Half of the chicks (randomly chosen, 10 chicks of each strain from each batch) were transported directly from the hatchery to the experimental unit and placed in their home pens, where they were given food and water. Transport of chicks from the hatchery to the laboratory began at approximately 15:00 h. The duration

of the short transport was approximately 4 h, and comprised transport by car to the research facilities after which chicks were weighed and placed into their home pens with immediate access to feed and water (SHORT). The rest of the chicks were food and water deprived for 10 h in addition to transport by car (LONG), during which time they were kept in a box and subjected to intermittent movement as a stress treatment. As previously stated in the introduction, the long transport treatment was meant to provide a standardized application of some of the conditions causing stress during transportation, including prolonged feed and water restriction, novelty, confinement and intermittent movement. The treatment was not meant to be a direct replication of the conditions under transportation. The duration of the long transport treatment was chosen partly because of the legislation, allowing transportation of chicks for up to 24 h and partly because of practice, in which chicks are transported for between 2 and 15 h. It was reasoned that if the 14 h treatment had detrimental effects on behavioural development, further studies should be done to test the effects of real transportation of different durations. On the other hand, if the long 14 h treatment had no detrimental effects, then transportation of this duration or lower would be unlikely to have adverse effects on behavioural development.

The movement was controlled using a laboratory shaker (SMI Multitube Vortexer; American Hospital Supply Corp., Miami, Florida, USA). The shaker produced an elliptical movement with the largest diameter measuring 3 cm. The movement took 0.5 s and was repeated every 3 s with a schedule of 15 min intermittent movement interspersed with 15 min of stillness. All chicks from the LONG treatment group had free access to feed and water directly after being introduced to their home pens following the stress treatment.

### 2.4. Video recordings

All pens were filmed from above using 24 h time-lapse during all light hours from arrival until day 19 after hatching.

### 2.5. Behaviour recordings

To determine when at least one chick in each pen was first seen on the perch, videos were observed continuously during all hours of light until the first perching observation was seen. This day was considered the day of first perching observation. After this, undisturbed perching behaviour was recorded in the home pens on days 6 and 11 after the first perching observation within each pen. The days were chosen to represent days when approximately 50% of the chicks can be expected to have started perching (day 6 after first perching observation) and when more or less all chicks should be using the perch (day 11 after first perching observation; Heikkilä et al., 2006). On both days the behaviour was recorded continuously during 2 h (10–11 and 14–15). In preliminary correlation analyses it was determined that these observational periods were representative of the entire light period. All behaviour recordings were done on pen level and included perching frequency and perching duration (mean bout duration and total duration). A perching observation was only recorded if it lasted for at least 5 s. Shorter perch visits were recorded as a separate variable (short perch visits).

### 2.6. Tonic immobility test

A tonic immobility (TI) test was performed on day 34 after hatching according to a modification of methods described by Jones (1986). The test was done in the room where the chicks were housed. Two observers performed the test simultaneously on different sides of the pens. They took one randomly chosen chick at a time from each pen. The next chick was taken from the neighbouring pen and after three chicks from each pen had been tested, the observers changed sides. During the test chicks were gently put on their back in a V-shaped wooden cradle. TI was induced by lightly covering the head with one hand and holding over the sternum with the other for 10 s. After this the hands were slowly removed and if the chick remained immobile for more than 10 s, the duration of TI was recorded. The maximum test duration was 600 s and no more than three induction attempts were made. Recordings included the number of induction attempts

needed to induce TI and the duration of TI (in seconds). After the TI test, chicks were weighed and marked with a numbered plastic leg ring for individual identification.

### 2.7. Food competition test

A food competition test was performed on day 36. Before the lights came on in the morning (at 08:30 h) the feeders were taken away from the pens and all litter and feed was removed from the floor. Testing was started after one hour of feed restriction. The two identical test pens (size: 60 cm × 60 cm) had wood chips on the floor and contained a metal feeder that allowed only one chick to eat at a time (Fig. 1.). One chick from each treatment and strain was taken out for each test, thus giving four chicks per test. The chicks were marked with a wax-based animal marker (DeLaval, Ski, Norway) to identify chicks of the same treatment. The order of marking was balanced over strains and treatments. Two tests were run simultaneously by two persons. During the 10 min test period scans were made by direct observation at 5 s intervals to record the identity of chicks with their head in the feeder. The variable used from this test was percentage of feeding observations per chick of all observations within the test group, which included one chick from each treatment within each strain.

### 2.8. Body weight of birds

Chicks were weighed directly after transport (immediately after arrival at the laboratory in SHORT birds and immediately after the simulated transport treatment in LONG birds), week 3 (day 20) and week 5 (day 34). Weights on day 1 and week 3 were recorded as pen averages, while chicks were weighed individually in week 5. For analyses of group-based variables, the group average was used also for week 5 weights.

### 2.9. Statistical analyses

The data was analysed using SPSS (Version 11.0). The variables were examined for normality and transformations were performed when necessary (Arcsin square root transformation for the competition test variables and LOG 10 transformation for the TI duration). The models for pen level perching behaviour and

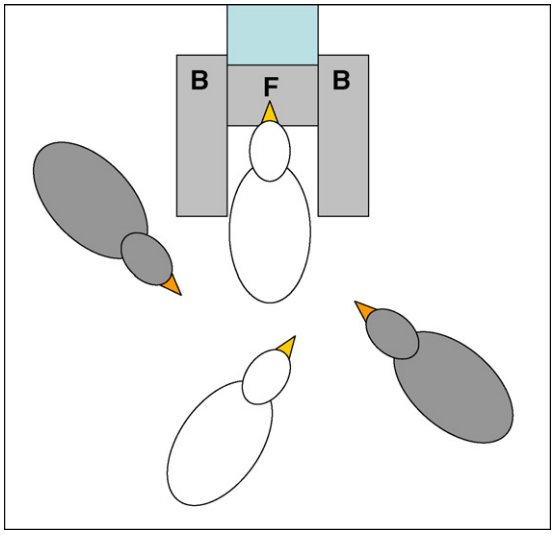


Fig. 1. A schematic picture of the pen used for the social competition test. *F* = Feeder, *B* = Bricks allowing only one bird to feed at a time.

weight and weight gain (mean weight for each pen) included strain and treatment as fixed effects and batch and pen position (front or back of room) as random effects. The models for TI and competition test results included strain and treatment as fixed effects and pen and batch as random effects. In these models the individual weight of the chicks on the day of the TI test was included as a covariate and for the TI test, test person was included as an additional random effect. Only results from final models, obtained by removing one variable (or interaction) at a time if it did not contribute significantly to the model, are reported. Descriptive statistics given are based on original, non-transformed values and include mean and standard deviation (S.D.). Tables also include median. The appropriateness of each test was considered by examining the normality of the residuals. Chi-square tests were used to determine effect of strain, treatment and test person on TI induction attempts.

## 3. Results

### 3.1. Weight and weight gain

There was no significant effect of strain or treatment ($P > 0.1$ for all) on weight of the chicks, even though HB birds were numerically heavier week 3 and week 5 than W-36 birds. Weight gain was not affected by treatment ($P > 0.1$ for all), but HB birds grew significantly faster than W-36 birds ($P < 0.001$; Table 1).

### 3.2. Perching behaviour

The chicks were first seen perching on average on day 6.2 (1.2) (mean and (S.D.)) after hatching. Perching frequency increased slightly from day 6 to day 11 after the day of the first perching observation, while perching bout duration almost doubled and total perching duration more than doubled during the same time period. There was a large variation in perching behaviour between groups (Table 2). The longer duration of transportation reduced the age at which chicks first were observed perching (SHORT: 6.8 (1.7), LONG: 5.6 (0.9), $F_{1,12} = 8.4$, $P = 0.01$). Strain did not influence when the first bird was seen perching ($P > 0.10$). The treatment had no effect on the frequency of perching on day 6 or 11 ($P > 0.10$). No effect of strain was found on perching frequency at day 6 ($P < 0.10$) but HB birds were observed to have a higher frequency of perching on day 11 than W-36 birds (45.5 (24.7) versus 24.8 (21.7), $F_{1,8} = 12.3$, $P = 0.008$). The treatment had no effect on the frequency of short perch visits ($P > 0.10$). W-36 birds had a higher frequency of short perch visits on day 6 than HB birds (9.1 (6.6) versus 2.8 (3.2), $F_{1,13} = 5.6$, $P = 0.03$) but this was not found on day 11 ($P > 0.10$). There was no effect of strain or treatment on mean perching bout duration or total perching duration,

Table 1
Weight and weight gain in grams and differences between strains at pen level ($n = 16$)

| | Hyline White-36 | | Hyline brown | | $P$ |
|---|---|---|---|---|---|
| | Mean (S.D.) | Median | Mean (S.D.) | Median | |
| Weight day 1 | 38.1 (5.45) | 38.2 | 39.6 (1.73) | 39.1 | >0.1 |
| Weight week 3 | 178 (9.56) | 176 | 207 (6.42) | 205 | >0.1 |
| Weight week 5 | 377 (21.5) | 371 | 456 (7.28) | 456 | >0.1 |
| Weight gain day 1 to week 3 | 140 (6.76) | 138 | 167 (5.95) | 168 | <0.001 |
| Weight gain week 3 to week 5 | 199 (13.1) | 197 | 249 (7.73) | 250 | <0.001 |
| Weight gain day 1 to week 5 | 339 (19.0) | 338 | 416 (6.87) | 418 | <0.001 |

Table 2
Descriptive statistics for perching behaviour on day 6 and 11 after first day of perching observed at 6.2 (1.2) (mean (S.D.)) days after hatching

| | Day 6 | | Day 11 | |
|---|---|---|---|---|
| | Mean (S.D.) | Median | Mean (S.D.) | Median |
| Perching frequency (observations/2 h) | 22 (20) | 19 | 35 (25) | 31 |
| Frequency (observations/2 h) of short perch visits (<5 s) | 6 (6) | 5.5 | 5 (5) | 3 |
| Mean perching bout duration/2 h (s) | 58 (28) | 62 | 103 (67) | 101 |
| Total perching duration/2 h (s) | 1578 (1799) | 1153 | 4124 (3340) | 3900 |

Data was collected at pen level ($n = 16$) from 10:00 to 11:00 h and from 14:00 to 15:00 h.

except for a tendency for LONG birds to have longer average perch bouts on day 6 ($F_{1,13} = 4.3$, $P = 0.06$) than SHORT birds (40.0 (30.2) versus 76.2 (38.6) s.).

### 3.3. TI-test

There was no effect of treatment on the TI duration ($P > 0.1$). However, there was a strong effect of strain (W-36: 416 (202) s, HB: 123 (108), $F_{1,74} = 48.8$, $P < 0.001$), W-36 having significantly longer TI duration than HB chicks. There was no effect of treatment or strain on the number of attempts necessary to induce TI ($P > 0.10$).

### 3.4. Competition test

The percentage of feeding observations per individual in the test group was affected by the treatment (SHORT: 30.6 (20.6), LONG: 21.9 (20.3), $F_{1,71} = 5{,}3$, $P = 0.02$) and the body weight of individual birds on day 34 ($F_{1,71} = 5.6$, $P = 0.02$). The treatment thus decreased the ability to compete, and heavier birds were better at competing. There was no effect of strain on the ability to compete for access to feed, however, there was an interaction between strain and treatment ($F_{1,71} = 5.7$, $P = 0.02$), showing that the effect of treatment was only apparent in the W-36 birds (W-36: SHORT: 25 (19) versus LONG: 7 (11), HB: SHORT: 36 (21) versus LONG: 36 (17)).

## 4. Discussion

Despite our expectation that simulated long transport might adversely affect growth and behavioural development only sporadic effects were found. There was no difference in weight or weight gain of birds nor in their fearfulness measured as the duration of TI between the two treatments. However, we did find that W-36 treated birds from the LONG treatment performed worse in the food competition test than birds exposed to the shorter duration of transport. In addition, and contrary to our hypothesis, LONG birds were observed to perch earliest.

The W-36 birds were expected to be more sensitive to the long transport treatment (see Webster, 1995), to be more fearful and to develop perch use earlier and use the perches more than the HB birds. The first two of these predictions were confirmed by the finding that the W-36 birds were more fearful, as indicated by a higher duration of TI, and more sensitive to the effects of simulated transport, as indicated by the fact that the treatment reduced competitive ability only in this strain. On the other hand, W-36 birds did not develop perching behaviour earlier or spend more time perching than the HB birds. The only strain difference in perching behaviour was a

higher frequency of short perch visits on day 6 for W-36 birds and a lower perching frequency for W-36 birds on day 11. The alleged strain differences in perching behaviour reported by producers were thus absent in the present experiment.

The exposure of birds to an additional 10 h of simulated transport in the present experiment was expected to cause chronic or intermittent stress in birds through a variety of mechanisms. In connection with the treatment of chicks we observed that LONG chicks made distress vocalizations during the times of movement but were completely quiet during periods of stillness. This suggests that chicks were deprived of sleep in addition to feed and water. On the other hand, the level of stress caused by the treatment cannot be ascertained as no independent measures were recorded. Another problem is that the treatment itself is confounded with the effects of experience, because the short transport chicks had earlier access to feed, water, visible conspecifics and perches than the long transport chicks. The observed effects may therefore be the result of a number of different factors or their interactions.

The finding that W-36 birds from the LONG treatment performed worse in the food competition test than W-36 birds exposed to the shorter duration of transport suggests that the ability of chicks to compete may be compromised by exposure to long durations of transportation during the first day of life. This could have implications for chick growth and survival under commercial conditions if adequate space for accessing feed and water are not provided during the first weeks of life. In contrast to the above, LONG birds of both strains were observed to perch earlier than SHORT birds, suggesting an earlier development of motor, cognitive or motivational factors related to perching. Although an earlier development of perching is likely to be adaptive, (Heikkilä et al., 2006), any suggested implications would be highly speculative as effects of the treatment on later perching behaviour were not observed. In conclusion, based on the number of observations and the lack of any effects of the treatment on growth the adverse effect of transporting chicks for 10–14 h appears to be marginal.

As we hypothesised, the W-36 strain was more fearful of humans as indicated by their longer durations of TI than the HB birds. This result corresponded well with our subjective experience that the W-36 birds were difficult to catch and handle, whereas the HB birds were easy to catch and appeared to be calm during handling. Because fear is a powerful stressor (Jones, 1997), these findings suggest that the W-36 birds were more stressed by contact with humans than the HB birds. Furthermore, competitive ability was reduced by the LONG treatment in W-36 but not in HB birds, also suggesting that stress sensitivity is higher in the former. Because the W-36 birds also have a slightly higher feed conversion ratio than the HB birds, the above-mentioned difference in stress sensitivity might be explained by resource allocation theory (Beilharz et al., 1993; Beilharz and Mitpaiboon, 1994). Empirical support for the idea that sensitivity to environmental changes increases in highly bred laying hens is provided by studies comparing red junglefowl and selected white leghorns (Lindqvist et al., 2002; Väisänen et al., 2005). On the other hand, the difference in feed conversion ratio between the W-36 and HB birds in the present experiment is so small that it might not have implications for coping with putative stressors.

## 5. Conclusions

A simulated long transport of day old chicks did not affect their body weight, growth, or the duration of tonic immobility in response to manual restraint, which was used as an indicator of fearfulness. Simulated long transport did reduce the ability of W-36 birds to compete for access to feed, suggesting that the W-36 birds were more sensitive to the effects of the simulated long transport as a putative stressor. This interpretation of the treatment effect on W-36 birds

corresponded well to the observation that this strain had a higher duration of tonic immobility than the HB birds. The simulated long transport treatment caused an earlier development of perching behaviour, but no other differences in perching behaviour were observed. Apart from the strain differences in tonic immobility and the treatment affect on competitive responses found for W-36 birds there were only sporadic and inconclusive differences in perching behaviour between the two strains of laying hens.

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